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PRIMER NOTE

Isolation and characterization of microsatellite loci in wheat stem sawfly *Cephus cinctus* and cross-species amplification in related species

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Abstract

The wheat stem sawfly is an important insect pest of wheat that can cause significant damage to yield and grain quality. Five microsatellite loci were isolated and characterized in wheat stem sawfly, *Cephus cinctus*, to facilitate future population genetic studies and help delineate their geographical origin. These loci were found to be polymorphic with an expected heterozygosity ranging from 0.304 to 0.937 and an observed heterozygosity ranging from 0.05 to 0.65. Successful cross-species amplification demonstrates the potential for these markers to provide a valuable tool for future population studies among related *Cephus* species.

Keywords: *Cephus cinctus*, cross-species amplification, microsatellite, wheat stem sawfly

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The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a key pest of wheat in the northern Great Plains of North America. Annual yield losses of $30 million have been reported from Montana, and region-wide losses, including several states and three Canadian provinces, may reach $100 million. In addition to feeding on wheat, sawfly larvae feed on rye, triticale, barley and a number of other grasses. First described as a pest in the 1890s, sawfly control strategies have focused on developing resistant cultivars and improved cultural practices. These approaches have been partially successful but have not been widely adopted. An alternative tactic, improving biological control by identifying and importing exotic natural enemies, has been initiated to complement these other control strategies. A recent study indicates that *C. cinctus* is present in eastern Asia, and could perhaps have a different suite of natural enemies than are present in North America (Ivie 2001). A study of *C. cinctus* genetic diversity was initiated to better understand the relationships among *Cephus* species and populations, to provide insight into the possible effectiveness of exotic natural enemies, and to better target foreign exploration activities. Microsatellite markers or simple sequence repeats (SSRs), were developed from enriched libraries of the wheat stem sawfly. This type of marker has been successfully characterized in Hymenoptera (Estoup *et al*. 1993) and shown to be valuable in population genetic studies, offering a high degree of allelic diversity (Bruford & Wayne 1993).

An optimized affinity capture technique described by Kandpal *et al*. (1994) was used to construct three libraries enriched for the dinucleotide repeats CT and GT, and a trinucleotide repeat TAA. Genomic DNA from sawfly larvae was digested with *Mbo*I restriction enzyme then ligated to *Mbo*I adapters. Digested fragments were heat denatured and allowed to hybridize to GT_{10}, CT_{10} or TAA_{12} biotin-labelled probes (Sigma-Genosys). Labelled probes were subsequently bound to streptavidin magnetic particles (Roche Molecular Biochemicals) as described by the manufacturer. The enriched microsatellite fragments were then cloned into pSTblue-1 (Perfectly Blunt Cloning Kit, Novagen). Plasmid DNA was isolated and inserts labelled for sequencing with the dye terminator cycle sequencing labelling reaction (Beckman Coulter) using either SP6 or T7 primers according to manufacturer's directions. Labelled templates were analysed with a CEQ 2000 DNA analysis system (Beckman Coulter). Sequence data from 135 microsatellite enriched clones suggested a 20% increase in SSRs over an

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Table 1 Primer sequences of five microsatellite loci from *Cephus cinctus*. Allele characteristics described are from the amplification of sixty individuals from populations of *C. cinctus* in Montana, North Dakota, Wyoming, Nebraska and Alberta Canada

Locus*	Repeat motif	Primer sequence 5′–3′ (fluorescent dye label)	Allele size (bp)	No. of alleles†	H_O‡	H_E§
SAWM28 (AY145889)	$(AG)_9AA(AG)_2$ $CGAG(AC)_2$	SAWM28F (D3) TTCACCCGAGCGTAACCTGTAT SAWM28R CGCGAAAATTCTAATGTGTCTC	149–171	10 (2)	0.533	0.720
SAWM44 (AY145890)	$(AAT)_3GAT$ $(AAT)_2$	SAWM44F (D2) CCGAGATGTTTTTCGGAGTT SAWM44R GGTTCTGGCATCGACTGAAT	215–219	4 (7)	0.050	0.304
SAWM46 (AY145886)	$(CT)_{16}$	SAWM46F (D3) GATCCCGGAAAGAAGGACTG SAWM46R CGTCACGTTTTATCGGAAGG	199–223	17 (8)	0.650	0.923
SAWM29 (AY145887)	$(CT)_{35}$	SAWM29F (D4) GCGAAAGGGTATCCTCATCC SAWM29R CCGTAAAAGAAGTGCAATCG	345–473	50 (16)	0.467	0.915
SAWM40 (AY145888)	$(TC)_{12}$	SAWM40F (D4) CCTCCCAATGAATGTGTACCAT SAWM40R GCTCGTTGTTAAGACTGTTTGC	229–259	25 (8)	0.600	0.937

*GenBank Accession nos. are given in parentheses.
†Number of individuals with null alleles given in parentheses.
‡Observed heterozygosity was calculated by including null alleles.
§Expected heterozygosity was calculated by including null alleles.
$H_E = 1 - \Sigma(x_i)^2$, (x_i = frequency of ith allele).

Table 2 Number of alleles found using *Cephus cinctus* primers with *Cephus pygmaeus*, *Cephus fumipennis* and *Trachelus tabidus* amplifications. The number of individuals used for amplification is in parentheses

Species	Collection location	*SAWM28*	*SAWM44*	*SAWM46*	*SAWM29*	*SAWM40*
Cephus fumipennis (8)	China	6	2	4	4	4
Cephus pygmaeus (4)	Turkey	5	1	1	4	3
Cephus pygmaeus (4)	Romania	4	0	1	4	2
Trachelus tabidus (1)	Turkey	2	1	1	2	1
Trachelus tabidus (5)	Romania	4	0	4	3	5

unenriched clone library. Primers were designed for 25 of these loci using the computer program PRIMER 3 (Rozen & Skaletsky 1998). The forward primers for 6 of the 25 primers were labelled with D4, D3 or D2 dyes (ResGen).

Genomic DNA was extracted from wheat stem sawfly individuals using DNeasy™ tissue extraction kit (Qiagen). The polymerase chain reaction (PCR) was performed in a 10-μL reaction volume containing 1× GeneAmp PCR buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% w/v gelatin), 0.2 mM each dNTP, 0.25 μM each primer, 50 ng template DNA, 1 U of AmpliTaq® DNA polymerase (Applied Biosystems). Amplification was performed in a Hybaid PCR Express thermal cycler (Thermo Hybaid) under the following conditions: 3 min at 94 °C (1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C) for 40 cycles, and 5 min at 72 °C. PCR products were then analysed using a fragment analysis program (Beckman Coulter).

Five microsatellite primers were used to analyse 60 sawfly individuals from 19 populations of *C. cinctus* in Montana, North Dakota, Wyoming, Nebraska and Alberta Canada. The microsatellite loci were found to be polymorphic with an expected heterozygosity ranging from 0.304 to 0.937 and an observed heterozygosity ranging from 0.05 to 0.65 (Table 1). Deviation of the observed heterozygosity from the expected heterozygosity may be due to the presence of null alleles in some individuals.

Polymorphic primers were then used to amplify other species of sawfly, *Cephus pygmaeus* and *Trachelus tabidus* from Turkey and Romania, and *Cephus fumipennis* from China (Table 2). With the exception of SAWM44, primers successfully amplified microsatellite loci from the three other sawfly species. These results suggest that the regions flanking these microsatellite loci may be sufficiently conserved to allow cross-species amplification and analysis in further studies.

The five microsatellite primers described here offer sufficient polymorphisms for future investigations into the genetic structure among the *C. cinctus* populations and possibly the relationships across species.

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